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Pharmacokinetics and pharmacodynamics of a novel protein kinase inhibitor, UCN-01

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Abstract *Purpose*: 7-Hydroxystaurosporine (UCN-01) is a potent protein kinase inhibitor and is being developed as a novel anticancer agent. We describe here its pharmacokinetics and pharmacodynamics in experimental animals. *Methods*: The pharmacokinetics of UCN-01 were studied following intravenous (i.v.) administration to mice, rats and dogs at doses of 1–9, 0.35–3.5 and 0.5 mg/kg, respectively. We also studied the pharmacodynamics of UCN-01 (9 mg/kg per day) during and after five consecutive i.v. administrations to nude mice bearing xenografted human pancreatic tumor cells (PSN-1). The concentrations of UCN-01 in plasma and tumor were measured by HPLC using a fluorescence detector. *Results*: UCN-01 in plasma after i.v. administration was eliminated biphasically in mice and rats, and triphasically in dogs. The elimination half-lives in mice, rats and dogs were 3.00–3.98, 4.02–4.46 and 11.6 h, respectively. The total clearance (Cl_{total}) values in mice, rats and dogs were high (1.93–2.64, 2.82–3.86 and 0.616 l/h per kg, respectively). The hepatic clearance ($Cl_{hepatic}$) in rats represented 54.0–81.3% of Cl_{total}. The volumes of distribution at steady-state in mice, rats and dogs were large (7.89–8.42, 13.0–16.9 and 6.09 l/kg, respectively). These pharmacokinetic parameters were dose-independent in mice and rats. UCN-01 produced significant inhibition of tumor growth during five consecutive i.v. administrations in mice bearing the xenografted PSN-1 cells, and the inhibitory effect continued for 3 days after the final administration. UCN-01 concentrations in tumor tissue were much higher than those in the plasma, and the ratio of tumor to plasma concentrations was about 500 at 24 h after five consecutive doses. *Conclusions*: The pharmacokinetic studies showed that UCN-01 has a high clearance and large distribution volume in various experimental animals, and its disposition is linear over the range of doses tested. The pharmacodynamic study showed that UCN-01 is distributed at much higher concentrations in tumor than those in plasma and that it significantly inhibits tumor growth. The high distribution of UCN-01 into tumor cells may contribute to the potent inhibition of tumor growth in vivo.

Key words 7-Hydroxystaurosporine (UCN-01) · Pharmacokinetics · Pharmacodynamics · HPLC

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Introduction

7-Hydroxystaurosporine (UCN-01, Fig. 1) has an indolocarbazole moiety and was originally isolated as a selective inhibitor of Ca^{2+}, phospholipid-dependent protein kinase (protein kinase C, PKC) [20]. UCN-01 has been shown to exhibit anticancer activity against human and murine tumor cell lines that have some aberrations in cellular signal transduction [1]. Unlike other compounds having the indolocarbazole moiety, UCN-01 preferentially induces G_1 phase accumulation in various cell lines [2, 5, 17], and it is clear that one of the mechanisms of action is mediated through the dephosphorylation of retinoblastoma protein and inhibition of cyclin-dependent kinase 2 (CDK2), an intracellular retinoblastoma protein kinase, which regulates the transition from the G_1 to S phase [6, 13]. In addition, UCN-01 enhances the anticancer effects of several important chemotherapeutic drugs, such as mitomycin C, cisplatin and 5-fluorouracil in vitro and in vivo [3, 4, 7, 16, 21]. Because of these unique mech-

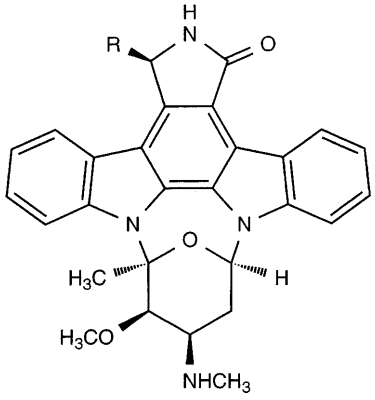

Fig. 1 Chemical structures of UCN-01 (R=OH) and staurosporine (R=H)

anisms of tumor growth inhibition, UCN-01 is expected to be effective in clinical use, and phase I clinical trials are ongoing in the US and Japan [11, 15].

Pharmacokinetics in laboratory animals are important in studying pharmacological and toxicological properties. To decide the starting dose in the phase I study, the toxic doses of UCN-01 in terms of body surface area in various animals were estimated. UCN-01, however, showed a large difference in the toxic doses among animal species. In practice, the starting dose in the phase I study was determined using the maximal tolerated dose in dogs, because the toxicity of the drug was more severe in dogs than in other experimental animals. However, since the anticancer activity was estimated in mice, comparison of the pharmacokinetic properties between mice and dogs was essential.

It has been reported that the activities of several anticancer drugs are dependent on the area under the plasma concentration versus time curves (AUC) [9]. On the other hand, the activities of anticancer drugs with a cell cycle phase-specific mechanism are dependent on the exposure time to the drugs, and these are called type II drugs [10]. The pattern of tumor growth inhibition by UCN-01 in a cell line might be exposure time-dependent [17], i.e. UCN-01 would be predicted to be a type II anticancer drug. The pharmacokinetics/pharmaco- or toxicodynamics with respect to the exposure time to the drug, e.g. the half-lives and the schedules, should be studied. Hence, the administration schedules of UCN-01 are very important to obtain sufficient efficacy in clinical use. In order to determine the optimal dosing schedule of UCN-01, pharmacokinetic studies in animals are indispensable. In addition, the concentrations of UCN-01 at the target sites, i.e. tumor cells, are directly relevant to the inhibitory activity. In the study reported here, we investigated the preclinical pharmacokinetics of UCN-01 after intravenous (i.v.) administration to mice, rats and dogs and the pharmacodynamics in nude mice bearing xenografted human pancreatic tumor cells (PSN-1).

Material and methods

Chemicals

UCN-01 and staurosporine were produced by fermentation in our Institute as described previously [20]. Acetonitrile (HPLC grade) was purchased from Kanto Chemical Company (Osaka, Japan). Other reagents used were of analytical grade and were obtained commercially.

Animals

Male BALB/c mice (Charles River Japan, Yokohama, Japan) weighing 25 to 27 g, male SD rats weighing 200 to 270 g (Japan SLC, Hamamatsu, Japan), and male HRP-MI male beagle dogs weighing 10 to 11.5 kg (HRP, Kalamazoo, Mich.) were used. PSN-1 human pancreatic carcinoma cells were obtained from the National Cancer Center Research Institute. The PSN-1 xenograft line was established by inoculation of cultured cells into male BALB/c-nu/nu mice weighing 21 to 28 g (Clea Japan, Tokyo, Japan). All animals were acclimated to a 12-h light/dark cycle at a controlled temperature (23 ± 1 °C) with free access to standard laboratory chow and water before the experiments. All experiments were approved by the Welfare Committee for Experimental Animals of our Institute.

Pharmacokinetics

For the pharmacokinetic studies of UCN-01, after bolus i.v. administration, the solutions were prepared by dissolving UCN-01 at appropriate concentrations in citric acid hydrate (1.2 mg/ml) with 5.6 mg disodium hydrogenphosphate dodecahydrate and 6.0 mg sodium chloride. UCN-01 at doses of 1, 3 and 9 mg/kg (maximum tolerated dose) was administered to mice via the tail vein. Under light ether anesthesia, mice were sacrificed, and blood samples were collected from the femoral artery and vein for immediate sampling at 0.083, 0.25, 0.5, 1, 2, 4, 6, 8, 12, 24, 48 and 72 h after administration and a small amount of heparin was added.

UCN-01 at doses of 0.35, 1 and 3.5 mg/kg was administered to rats via the femoral vein. Each rat was kept in a supine position on a board for drug administration and allowed to recover from light ether anesthesia prior to the injection of drugs. It took a few minutes for the animals to recover from the anesthesia. The animals were released from restraint after dosing. Blood samples (0.1–0.25 ml) were collected into heparinized capillary tubes (Drummond Scientific Company, Broomall, Pa.) from the tail vein of the rats restrained in a Ballman cage (Natsume Seisakusho Company, Tokyo, Japan) at 0.083, 0.25, 0.5, 1, 2, 4, 8, 12, 24, 36 and 48 h after dosing. UCN-01 was also administered at a dose of 0.5 mg/kg to dogs via the foreleg vein. Blood samples (5 ml) were collected from the contralateral foreleg vein using a heparinized disposable syringe at 0.083, 0.25, 0.5, 1, 2, 4, 6, 8, 12 24, 48, 72 and 96 h after dosing. All experiments were conducted with three animals in each experimental group. Plasma was obtained by blood centrifugation (1500 *g*) for 15 min, and stored below −70 °C until assayed.

Hepatic extraction

Hepatic extraction in rats ($n = 3$) was measured by the method of Yokota et al. [23]. Briefly, under light ether anesthesia, the abdomen of the rats was opened by a midline incision downwards extending about 4 cm from the diaphragm. The animals were anesthetized for less than 30 min during the entire surgical procedure. As a cannula, a venous injection needle (0.65 × 25 mm; Terumo, Tokyo, Japan) was used, bent at a right-angle 3.5 mm from the tip connected to a PE-50 polyethylene tube (Becton Dickinson, Parsippancy, N.J.). This cannula was connected to a 1-ml syringe and then the bent needle tip of the cannula was inserted

downwards into the injection site of the hepatic vein. The cannulated site was protected by a surgical binding agent (Aron Alpha, Sankyo Company, Tokyo, Japan), and then the abdomen was sutured. Femoral arterial and venous cannulation was carried out by the usual methods with PE-50 polyethylene tubing (0.5 mm i.d.). After recovery from anesthesia, the drug was infused via the femoral venous cannula using an automatic microinfusion pump (model 975, Harvard Apparatus, South Natick, Ma.). The infusion rate was 0.065 ml/min. The infusion started after bolus administration of UCN-01 at doses of 0.06, 0.6 and 3 mg/kg, and the infusion dose rates of UCN-01 were 0.5, 5 and 25 μg/kg per min. Since the plasma concentrations of UCN-01 were kept constant between 20 and 40 min, blood samples of 250 μl from the femoral artery and hepatic vein of each rat were collected into heparinized capillary tubes at 20, 30 and 40 min after infusion. Plasma samples were separated and stored below −70 °C until assayed.

In order to calculate rat hepatic clearance, the blood-plasma distribution ratio (Rb value) was estimated using rat blood in vitro. UCN-01 was added to heparinized blood of rats not treated with drug, and the final UCN-01 concentration was prepared at 500 ng/ml. After incubation at 37 °C, the plasma concentration (Cp) obtained by centrifugation was measured. The Rb value was calculated from the following equation:

$$\mathrm{Rb} = \frac{\mathrm{Cb}}{\mathrm{Cp}} \tag{1}$$

where Cb is the blood concentration.

Pharmacodynamics

The concentrations of UCN-01 in plasma and tumor, when UCN-01 produced tumor growth inhibition after dosing, were studied in nude mice bearing xenografted PSN-1. PSN-1 tumors (about 8-mm^3 fragments) were inoculated subcutaneously into the flanks of nude mice. Treatment with UCN-01 was initiated 2 weeks after the tumors were implanted. UCN-01 was administered to nude mice by five consecutive i.v. administrations via the tail vein at a dose of 9 mg/kg. Under light ether anesthesia, blood samples were collected from the femoral artery and vein at 0.083, 1, 2, 4, 8, 12 and 24 h after the first dose, at 24 h after the second to fourth doses which was just prior to the next dose, and at 0.083, 1, 2, 4, 8, 12, 24, 48, 72 and 120 h after the fifth dose. Plasma samples were obtained by centrifugation of the blood. Simultaneously, the tumor tissues were removed. The samples of plasma and tumor were stored below −70 °C until assay. For evaluation of the anticancer activity of UCN-01, tumor volumes were calculated from the following equation, according to the method of the National Cancer Institute [8], from the length and width of the tumors measured in millimeters:

$$\text{Tumor volume } (\mathrm{mm}^3) = \frac{\text{length} \times \text{width}^2}{2} \tag{2}$$

Drug efficacy is expressed as the mean V/V_0 value expressed as a percentage in relation to that of the control group not treated with drugs, where V is the tumor volume on the day of evaluation and V_0 is the tumor volume on the day of the initial treatment with the drug. The tumor volumes at the start of treatment ranged from 381 mm^3 to 1290 mm^3.

Sample treatment

According to the previously described method for determining UCN-01 in human plasma [14], UCN-01 concentrations in animal plasma and tumor tissue were measured by HPLC using a fluorescence detector. For the determination of UCN-01 in plasma, a plasma sample was deproteinized by adding twice the volume of ice-cold acetonitrile solution containing staurosporine as an internal standard (50 ng/ml). After deproteinization, the mixture was vigorously vortexed for 30 s and then stood on ice for 10 to 15 min in an ice box with a cover because UCN-01 is sensitive to light and degrades when left under natural light. After centrifugation (10 000 to 11 000 *g* for 10 min at 4 °C) of the mixture, an aliquot (100 μl) of the supernatant was injected onto an HPLC system. For determination of UCN-01 in tumor tissue, ice-cold 50 m*M* phosphate buffer (pH 6.3), nine times the volume of the dissected tumor, was added to the tumor, and then the mixture was homogenized. The tumor homogenate obtained was deproteinized by adding twice the volume of acetonitrile, as for the plasma. After centrifugation, the supernatant was passed through a filter membrane (0.22 μm), and an aliquot of the filtrate was injected onto the HPLC system.

HPLC conditions

The concentration of UCN-01 was determined using an HPLC system with a fluorescence detector (excitation wavelength 310 nm, emission wavelength 410 nm) as previously reported [14]. Briefly, the column was an AM-312 (ODS) reverse-phase column (6 mm ID × 150 mm; YMC Company, Kyoto, Japan), the mobile phase was acetonitrile/0.1% triethylamine in 50 m*M* phosphate buffer (pH 7.3), and the flow rate was 1 ml/min. Standard curves were prepared using blank plasma or tumor homogenate from other animals not treated with the drug. The concentration ranges of the standard curves were 0.2 to 300 ng/ml in plasma and 2 to 1000 ng/g in tumor. UCN-01 concentrations in the samples were determined from standard curves obtained by plotting the peak ratio (drug/internal standard, y) versus drug concentration. The curves were determined by linear regression analysis with a weighting factor of $1/y^2$. There were no interfering peaks in any blanks, and the standard curves showed good linearity. The precision at the lower limit of quantitation (LLOQ, 0.2 ng/ml in plasma and 2 ng/g in tumor) was within 11.0%, and the accuracy was within ±11.8% at any concentration. The intra- and interday assay precision at the lower concentrations, but not the LLOQ, were within 15.6%, and those at middle or higher concentrations were within 15%. The values of accuracy were within ±15%.

Pharmacokinetic analysis

Pharmacokinetic parameters were estimated model-independently [12, 22]. The plasma or tumor concentration was converted to a logarithm and plotted against time, and then the slope (elimination rate constant, k) of the terminal phase was calculated by fitting using the linear least squares method. The elimination half-life ($t_{1/2}$) was calculated as 0.693/k, and the area under the plasma and tumor AUCs were calculated by the trapezoidal method. The AUC infinity was calculated by extrapolation using k. Total plasma clearance (Cl_{total}) was calculated as dose/AUC. Mean residence time (MRT) and steady-state volume of distribution (Vdss) were calculated from the following equations:

$$\mathrm{MRT} = \frac{\int_0^\infty \mathrm{t} \times \mathrm{Cp}\ \mathrm{dt}}{\int_0^\infty \mathrm{Cp}\ \mathrm{dt}} \tag{3}$$

$$\mathrm{Vdss} = \mathrm{Cl_{total}} \times \mathrm{MRT} \tag{4}$$

where t is the time and Cp is the concentration at time t.

To estimate the rat hepatic blood clearance ($Cl_{hepatic}$), the hepatic extraction ratio (ER) was calculated using the femoral artery plasma concentration ($C_{ss,a}$) and the hepatic vein plasma concentration ($C_{ss,v}$) at steady-state from the following equation:

$$\mathrm{ER} = \frac{\mathrm{C_{ss,a}} - \mathrm{C_{ss,v}}}{\mathrm{C_{ss,a}}} \tag{5}$$

$Cl_{hepatic}$ was then calculated from the following equation:

$$\mathrm{Cl_{hepatic}} = \mathrm{Q_{hepatic}} \times \mathrm{ER} \tag{6}$$

where $Q_{hepatic}$ is the hepatic blood flow rate (60.5 ml/min per kg) [23]. The total blood clearance at steady-state ($Cl_{total,ss}$) was then calculated from the following equation:

$$Cl_{total,ss} = \frac{I_0}{C_{ss,a} \times Rb} \quad (7)$$

where I_0 is the infusion dose rate and Rb is the blood-plasma distribution ratio (see Eq. 1).

Statistical analysis

Statistical analysis was conducted using the SAS system release 6.11 for Windows (SAS Institute Japan, Tokyo, Japan). A comparison of the pharmacokinetic parameters among doses in rats was carried out by one-way ANOVA when the homogeneity of variance in the data was confirmed by the Bartlett test, and by the Steel-Dwass test when there was no homogeneity of variance in the data. The testing for significant differences between tumor growth in the UCN-01-treated group and that in the control group with no drug treatment was carried out using the unpaired Student's *t*-test (two-tailed), $P < 0.05$ being considered significant.

Results

Pharmacokinetics

The plasma concentration versus time profiles of UCN-01 after bolus i.v. administration to mice, rats and dogs are shown in Fig. 2A–C, respectively. The plasma concentrations at doses of 1, 3 and 9 mg/kg in mice, and at doses of 0.35, 1 and 3.5 mg/kg in rats rapidly decreased biphasically, while the plasma concentrations at a dose of 0.5 mg/kg in dogs decreased triphasically and more slowly compared with those in mice and rats. The pharmacokinetic parameters are shown in Table 1. The AUCs of UCN-01 in mice after i.v. administration increased in proportion to the dose in the range 1 to 9 mg/kg. The dose-independent parameters, such as $t_{1/2}$, Cl_{total}, Vdss and MRT, were almost equal among doses, and the values of Cl_{total} and Vdss were relatively high. As in mice, the AUCs of UCN-01 in rats after bolus i.v. administration increased in proportion to the dose in the range 0.35 to 3.5 mg/kg, and there was no significant difference between the dose-independent parameters. The values of Cl_{total} and Vdss were also high. The elimination of UCN-01 in dogs was slower than in mice and rats, and the $t_{1/2}$ was 11.6 ± 1.7 h. The Cl_{total} (0.616 ± 0.235 l/h per kg) was one-sixth to one-third of that in mice and rats. The Vdss in dogs was also large (6.09 ± 1.98 l/kg).

Hepatic extraction during infusion of UCN-01 in rats was evaluated (Table 2). The plasma concentrations in both the femoral artery and the hepatic vein at 20 to 40 min after the start of infusion almost reached a steady state (data not shown). The $Cl_{total,ss}$ was almost equal to that after bolus administration. The ER was found to be constant and independent of the infusion rate, and was 0.405 ± 0.189 to 0.558 ± 0.139. The $Cl_{hepatic}$ calculated from the ER obtained was 1.47 ± 0.69 to 2.03 ± 0.50 l/h per kg, and this accounted for about 50% to 80% of the $Cl_{total,ss}$.

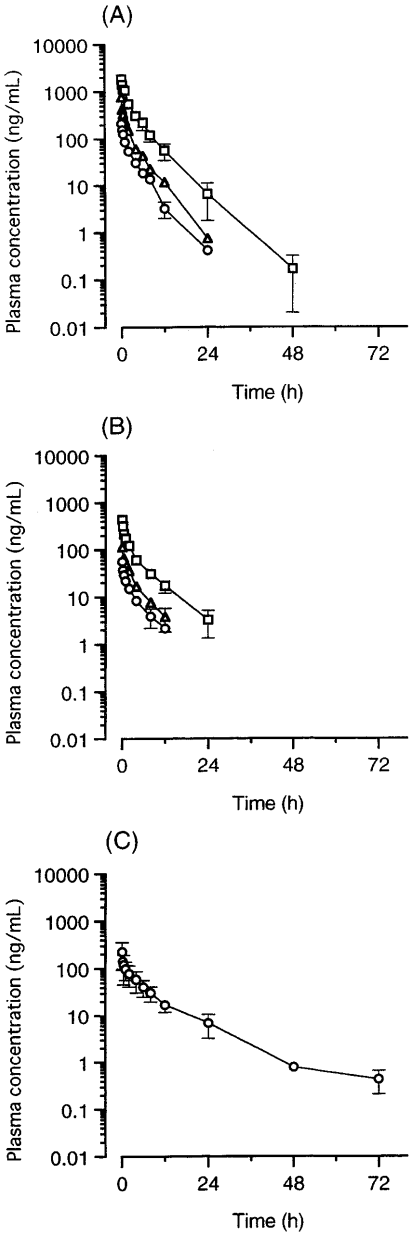

Fig. 2A–C Plasma concentration versus time profiles of UCN-01 after i.v. administration to mice at doses of 1 (*circles*), 3 (*triangles*) and 9 (*squares*) mg/kg (**A**), to rats at doses of 0.35 (*circles*), 1 (*triangles*) and 3.5 (*squares*) mg/kg (**B**) and to dogs at a dose of 0.5 mg/kg (**C**) Each point represents the mean ± s.d. from three animals

Pharmacodynamics

The profile of the growth-inhibitory activity of UCN-01 in nude mice bearing xenografted PSN-1 tumors during and after five consecutive i.v. administrations of UCN-01 is shown in Fig. 3. During the dosing, UCN-01 produced significant inhibition of tumor growth compared with the control group with no drug treatment and its inhibitory effect continued until the 3rd day after the end of the five consecutive doses. The plasma and

Table 1 Pharmacokinetic parameters of UCN-01 after i.v. administration at the doses indicated to mice, rats and dogs

	$t_{1/2\alpha}$ (h)	$t_{1/2\beta}$ (h)	$t_{1/2\gamma}$ (h)	$AUC_{0-\infty}$ (ng · h/ml)	Cl_{total} (l/h/kg)	Vdss (l/kg)	MRT (h)
Mice							
1 mg/kg	0.405	3.11	–	432	2.31	7.89	3.41
3 mg/kg	0.399	3.00	–	1130	2.64	8.42	3.19
9 mg/kg	0.884	3.98	–	4670	1.93	7.95	4.13
Rats[a]							
0.35 mg/kg	0.542 ± 0.213	4.09 ± 1.03	–	125 ± 11	2.82 ± 0.24	13.0 ± 2.7	4.66 ± 1.13
1 mg/kg	0.842 ± 0.067	4.02 ± 1.88	–	262 ± 35	3.86 ± 0.55	16.7 ± 4.9	4.45 ± 1.79
3.5 mg/kg	0.560 ± 0.178	4.46 ± 0.80	–	1019 ± 162	3.49 ± 0.52	16.9 ± 0.4	4.92 ± 0.90
Dogs[a]							
0.5 mg/kg	0.142 ± 0.057	2.99 ± 0.55	11.6 ± 1.7	925 ± 444	0.616 ± 0.235	6.09 ± 1.98	10.1 ± 0.8

[a] Each value represents the mean ± s.d. from three animals

Table 2 Hepatic extraction ratio and hepatic blood clearance of UCN-01 in rats. Each value is the mean ± s.d. from three rats ($C_{ss,a}$ femoral artery plasma concentration at steady state, $C_{ss,v}$ hepatic vein plasma concentration at steady state)

Parameter	Infusion rate (μg/kg/min)		
	0.5	5	25
$C_{ss,a}$ (ng/ml)	5.59 ± 0.14	61.1 ± 2.9	225 ± 44
$C_{ss,v}$ (ng/ml)	3.31 ± 0.96	26.8 ± 7.7	108 ± 40
Hepatic extraction ratio (ER)	0.405 ± 0.189	0.558 ± 0.139	0.518 ± 0.172
Total blood clearance ($Cl_{total,ss}$)[a] (l/h/kg)	2.74 ± 0.07	2.51 ± 0.12	3.48 ± 0.63
Hepatic blood clearance ($Cl_{hepatic}$) (l/h/kg)	1.47 ± 0.69	2.03 ± 0.50	1.88 ± 0.63
$Cl_{hepatic}/Cl_{total,ss}$ ratio	0.540 ± 0.260	0.813 ± 0.225	0.554 ± 0.208

[a] Blood-plasma distribution ratio = 1.96

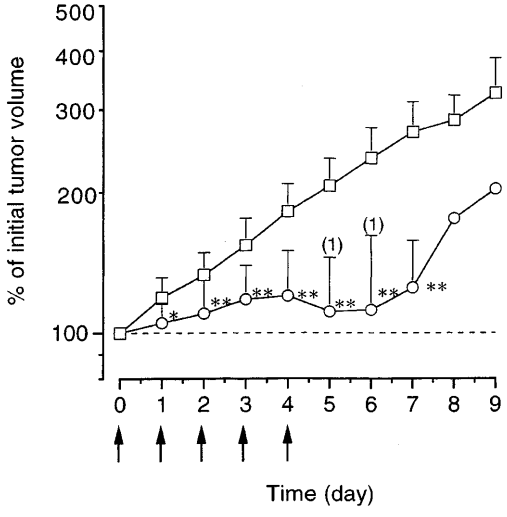

Fig. 3 Tumor proliferation in the control group of nude mice not treated with drug (**$P < 0.01$) (*squares*) and the UCN-01-treated group of nude mice (*circles*) bearing xenografted PSN-1 (human pancreatic carcinoma cells) after five consecutive i.v. administrations of UCN-01 at a dose of 9 mg/kg per day. The time-points for days 1, 2, 3 and 4 were just prior to the next dose. Each point represents the mean ± s.d. ($n = 4$ to 9) or mean ($n = 2$). The *arrows* indicate the times of i.v. administration of UCN-01. The figures in parentheses are the number of animal deaths (*$P < 0.05$, **$P < 0.01$)

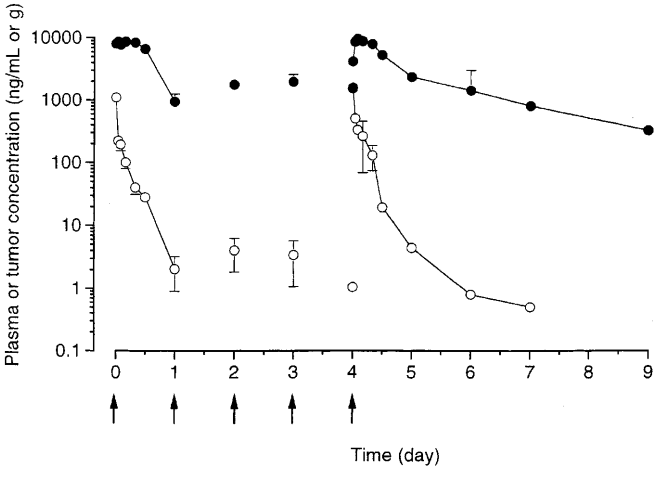

Fig. 4 Plasma (*open circles*) and tumor (*closed circles*) concentration versus time profiles of UCN-01 after five consecutive i.v. administrations of UCN-01 at a dose of 9 mg/kg per day to nude mice bearing xenografted PSN-1 (human pancreatic carcinoma cells). Each point represents the mean ± s.d. ($n = 3$) or mean ($n = 2$). The *arrows* indicate the times of i.v. administration of UCN-01

tumor concentration versus time profiles and pharmacokinetic parameters of UCN-01 are shown in Fig. 4 and Table 3, respectively. The plasma concentrations in nude mice after dosing rapidly decreased in a biphasic manner. The plasma concentration on day 9 was not detectable (<0.2 ng/ml). Tumor concentrations were much higher than those in plasma and the elimination half-life of UCN-01 from tumor (4.85 h on day 1) was slightly longer than that in plasma (3.48 h). The plasma and tumor concentration versus time profiles after

Table 3 Pharmacokinetic parameters of UCN-01 in plasma and tumor after single and five consecutive i.v. administrations (multiple) of UCN-01 (9 mg/kg per day) to nude mice bearing xenografted PSN-1 (human pancreatic carcinoma) (– not determined)

Parameters	Plasma		Tumor	
	Single	Multiple	Single	Multiple
$t_{1/2\alpha}$ (h)	0.168	0.245	–	–
$t_{1/2\beta}$ (h)	3.48	3.11	4.85	–
$t_{1/2\gamma}$ (h)	–	35.4	–	34.3
AUC_{0-24h} (ng · h/ml)	1820	3410	143 000	139 000
$AUC_{0-\infty}$ (ng · h/ml)	–	–	–	253 000
Cl_{total} (l/h/kg)	–	2.64	–	–
MRT (h)	–	4.09	–	–
Vdss (l/kg)	–	10.8	–	–

multiple dosing were almost identical to those after the first dose, indicating little accumulation of UCN-01 in the body. The AUCs in plasma until 24 h after the first and fifth doses were 1820 and 3410 ng · h/ml, respectively, and those in tumor were 143 000 and 139 000 ng · h/g tissue, respectively.

Discussion

To investigate its disposition in various experimental animals, UCN-01 was administered i.v. to mice, rats and dogs. The plasma concentrations in mice and rats rapidly decreased biphasically, while those in dogs decreased in a triphasic manner and more slowly compared with those in mice and rats. The AUCs in mice and rats increased in proportion to the dose administered. Thus, UCN-01 showed linear pharmacokinetic profiles in mice and rats for the doses tested. The values of Cl_{total} in rats were almost equal to their hepatic blood flow. Furthermore, the hepatic blood clearance of UCN-01 made a major contribution to its total blood clearance. These results suggest that UCN-01 is eliminated mainly via the liver. The elimination ($t_{1/2}$, MRT) of UCN-01 in dogs was slower than in mice and rats. This might correlate with the fact that the toxicity of UCN-01 following rapid injection in dogs was more severe than that in the other test animals (data not shown). Considering these results, the toxicity might be related to the exposure time-dependent anticancer activity of UCN-01. The protein binding of UCN-01 is very high in the serum of various species, and the ratio of the free to total fraction has been found to be below 0.3% by the ultrafiltration method (data not shown). Since the compound binds strongly to plasma protein and it is hard to estimate accurately the unbound fraction, the precise determination of pharmacokinetic parameters is difficult. UCN-01 binds strongly to serum protein, while the volume of distribution at steady-state in various experimental animals is relatively large. These findings suggest that UCN-01 also binds to tissue proteins.

The anticancer activity of UCN-01 following i.v. administration was assessed in nude mice bearing xenografted PSN-1 cells. UCN-01 showed significant inhibitory activity against tumor growth and its effect lasted after drug administration had ceased. In this pharmacodynamic study, the tested dose (9 mg/kg per day for 5 consecutive days) is the maximum tolerated dose in male BALB/c mice (unpublished data). The plasma pharmacokinetic profile in xenografted nude mice was almost identical to that in normal mice. The plasma clearance in nude mice was also high and the plasma concentrations were much lower than the tumor concentrations. The ratio of tumor to plasma concentration (T/P ratio) became high immediately after dosing. Although this might suggest the existence of a transport mechanism for UCN-01 to PSN-1 cells, this is not yet clear. The high T/P ratio may contribute to the potent in vivo anticancer activity. On the other hand, it is important for the prediction of UCN-01 toxicity to consider the distribution to normal tissues as well as to tumor cells. Although it is necessary to verify the efficacy or toxicity of UCN-01 in humans, UCN-01 appears to be a very attractive drug for cancer chemotherapy.

Depending on the mechanism responsible for their cytotoxicity to tumor cells, anticancer drugs can be classified into two types: type I (cell cycle phase nonspecific) and type II (cell cycle phase specific) [10, 18]. The cell growth inhibitory activity of type I or II drugs depends on the product of concentration and time (AUC) in the culture medium or plasma, or the exposure time [10]. The anticancer activity of UCN-01 against several tumor cells evaluated for various exposure times exhibits a time-dependence [17]. This may be explained by inhibition of the signaling pathways in the cell cycle. However, when tumor cells are exposed for a long enough period, the activity of UCN-01 seems to show AUC dependence [17]. The elimination half-lives of UCN-01 after multiple doses to nude mice bearing PSN-1 cells were relatively long. The in vivo efficacy could depend on the AUC.

The tumor appeared to regrow on day 9 when the tumor UCN-01 concentration was 324 ng/g ($n = 2$). The precise mechanism for this regrowth was not clear, but there might be a concentration threshold for the antiproliferative activity of UCN-01 in vivo. As described above, the half-lives of several anticancer drugs in humans are longer than in mice [19]. It may be presumed that the AUC is a good predictor of the efficacy and toxicity of UCN-01 in clinical studies. The large volumes of distribution of UCN-01 suggest high distribution into tissues. Indeed, higher radioactivity in various tissues than in plasma has been found after i.v. administration of tritium-labeled UCN-01 to rats (unpublished data).

In conclusion, UCN-01 had a high plasma clearance and a large volume of distribution at steady-state in animals, and linear pharmacokinetics over the dose ranges tested. The elimination in rats took place mainly

via the liver. The extensive distribution to tumor cells may contribute to the potent anticancer activity of UCN-01.

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